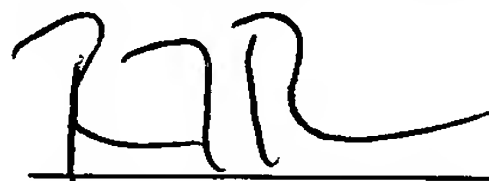


Therefore, no new matter has been added. Entry of the above amendment and examination of the application are respectfully requested.

\* \* \*

Respectfully submitted,  
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By:



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SEP 06 2002

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE TECH CENTER 1600/2900

Applicant: Van Rooijen t al.

Serial No.: 10/032,201

Filed: December 19, 2001

For: **METHODS FOR THE  
PRODUCTION OF MULTIMERIC  
PROTEINS COMPLEXES, AND  
RELATED COMPOSITIONS**

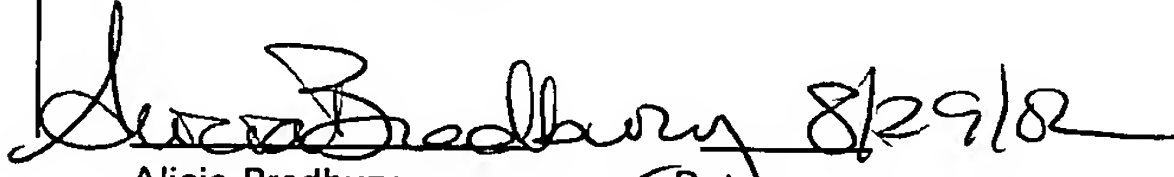
Art Unit: 1638

Examiner: Unassigned

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Alicia Bradbury  
Date 8/29/02

**ATTACHMENT TO THE PRELIMINARY AMENDMENT  
MARKED UP PARAGRAPHS AND CLAIMS**

U.S. Patent and Trademark Office  
P.O. Box 2327  
Arlington, VA 22202

Dear Sir:

Preliminary to examination of the above-captioned application, please  
amend the application as follows:

**IN THE SPECIFICATION:****Please amend the paragraph on page 1, lines 23-28, as follows;**

Multimeric proteins (i.e. proteins [compising] comprising multiple  
polypeptide chains) are a biologically and commercially important class of  
proteins. Antibodies for example are multimeric proteins which are used to treat  
a wide range of disease conditions. However in view of their complexity,  
multimeric proteins frequently represent significant manufacturing challenges.

**Please amend the paragraph on page 2, lines 11-14, as follows;**

However, there is a need in the art to further improve the methods for the  
recombinant expression of multimeric proteins, including redox proteins. The  
present invention [satisfies] satisfies this need and provides related advantages

as well.

**Please amend the paragraph on pages 2, line 30, to page 3, line 30, as follows;**

The method further contemplates isolating the oil bodies associated with said recombinant multimeric-protein-complex. The second recombinant polypeptide can be associated with a second oil-body-targeting-protein capable of associating with an oil body and said second recombinant polypeptide. Each of said oil-body-targeting-proteins can be [a] an oil-body-protein or an immunoglobulin. The oil-body-targeting-protein can be an oleosin or caleosin. When the oil-body-targeting-protein can be an oleosin or caleosin, the first recombinant polypeptide can be fused to said oleosin or caleosin. Likewise, the second recombinant polypeptide can be fused to a second oleosin or second caleosin capable of associating with an oil body. The first and second recombinant polypeptides can be produced as a multimereic-fusion-protein comprising said first and second [polypeptide] polypeptide, and can form a multimeric-protein-complex. The multimeric-protein-complex can be a heteromultimeric-protein-complex, and the heteromultimeric-protein-complex can be an enzymatically active redox complex or an immunoglobulin. In one embodiment, the first recombinant polypeptide is capable of associating with said second recombinant polypeptide in the cell. In another embodiment, the first recombinant polypeptide can be a thioredoxin and the second recombinant polypeptide can be a thioredoxin-reductase. In particular embodiments, the thioredoxin can be selected from the group consisting of SEQ ID NOs:38, 42, 46, 50 and SEQ ID NOs:52-194; and the thioredoxin-reductase can be selected from the group consisting of those set forth in SEQ ID NOs:8, 9, 10, 40, 44, 48, 50 and SEQ ID NOs:195-313.[^C] In another embodiment, the first recombinant polypeptide can be an immunoglobulin-polypeptide-chain. For example, the first recombinant polypeptide can be an immunoglobulin light chain, or an immunologically active portion thereof, and the second recombinant polypeptide can be an immunoglobulin heavy chain, or an immunologically active portion thereof. In this embodiment, the oil-body-targeting-protein can comprise

protein A, protein L or protein G. The cell can be a plant cell, such as a safflower cell, and the like.

**Please amend the paragraphs on page 4, line 20, to page 5, line 24, as follows;**

(d) associating said first recombinant polypeptide with an oil body through an oil-body-targeting-protein capable of associating with said oil bodies and said first recombinant polypeptide. This method further contemplates isolating from the progeny cell, oil bodies comprising the multimeric-protein-complex. The second recombinant polypeptide can be associated with a second oil-body-targeting-protein capable of associating with an oil body and second recombinant polypeptide. Each of said oil-body-targeting-proteins can be [a] an oil-body-protein or an immunoglobulin. The oil-body-targeting-protein can be an oleosin or caleosin. When the oil-body-targeting-protein is an oleosin or caleosin, the first recombinant polypeptide can be fused to said oleosin or caleosin. Likewise, the second recombinant polypeptide can be fused to a second oleosin or second caleosin capable of associating with an oil body. The first and second recombinant polypeptides can be produced as a [multimereic] multimeric-fusion-protein comprising said first and second [polypetide] polypeptide, and can form a multimeric-protein-complex. The multimeric-protein-complex can be a heteromultimeric-protein-complex, and the heteromultimeric-protein-complex can be an enzymatically active redox complex or an immunoglobulin. In one embodiment, the first recombinant polypeptide and said second recombinant polypeptide are capable of forming a multimeric-protein-complex in said progeny cell. In another embodiment, the first recombinant polypeptide can be a thioredoxin and the second recombinant polypeptide can be a thioredoxin-reductase. In particular embodiments, the thioredoxin can be selected from the group consisting of SEQ ID NOs:38, 42, 46, 50 and SEQ ID NOs:52-194; and the thioredoxin-reductase can be selected from the group consisting of those set forth in SEQ ID NOs:8, 9, 10, 40, 44, 48, 50 and SEQ ID NOs:195-313. In another embodiment, the first recombinant polypeptide can be an immunoglobulin-polypeptide-chain. For example, the first

recombinant polypeptide can be an immunoglobulin light chain, or an immunologically active portion thereof, and the second recombinant polypeptide can be an immunoglobulin heavy chain, or an immunologically active portion thereof. In this embodiment, the oil-body-targeting-protein can comprise protein A, protein L or protein G. The cell can be a plant cell, such as a safflower cell, and the like.

**Please amend the paragraphs on page 7, line 8, to page 9, line 30, as follows;**

(c) a third nucleic acid sequence encoding a second recombinant polypeptide, wherein said first and second recombinant polypeptide are capable of forming a multimeric-protein-complex. The oil-body-targeting-protein can be selected from an oil-body-protein or an immunoglobulin. The oil-body-protein can be an oleosin or caleosin. The multimeric-protein-complex can be a heteromultimeric-protein-complex, and the first and second recombinant polypeptide can form an enzymatically active heteromultimeric redox complex or an immunoglobulin. In a particular embodiment, the first recombinant polypeptide is a thioredoxin and the second recombinant polypeptide is a thioredoxin-reductase. The thioredoxin can be selected from the group consisting of SEQ ID NOs:38, 42, 46, 50 and SEQ ID NOs:52-194; and the thioredoxin-reductase can be selected from the group consisting of those set forth in SEQ ID NOs:8, 9, 10, 40, 44, 48, 50 and SEQ ID NOs:195-313. In another embodiment, the first recombinant polypeptide can be an immunoglobulin-polypeptide-chain. For example, the first recombinant polypeptide can be an immunoglobulin light chain, or an immunologically active portion thereof, and the second recombinant polypeptide can be an immunoglobulin heavy chain, or an immunologically active portion thereof. In this embodiment, the oil-body-targeting-protein can comprise protein A, protein L or protein G. In yet another embodiment, positioned between the nucleic acid sequence encoding an oil-body-targeting-protein and the nucleic acid sequence encoding a first recombinant polypeptide can be a linker nucleic acid sequence encoding [a] an oil-body-surface-avoiding linker amino acid sequence. The oil-body-surface-avoiding linker amino acid sequence can be substantially

negatively charged, or have a molecular weight of at least 35 kd. Optionally, the gene fusion further comprises a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by an enzyme or a chemical, wherein the linker sequence is positioned between the oil-body-surface-avoiding linker amino acid sequence that is also a non-proteolytic linker and said sequence encoding the first recombinant polypeptide.

Also provided herein are recombinant multimeric-fusion-proteins comprising (i) an oil-body-targeting-protein, or fragment thereof, (ii) a first recombinant polypeptide and a (iii) second recombinant polypeptide, wherein said first and second recombinant polypeptides are capable of forming a multimeric-protein-complex. The oil-body-targeting-protein can be selected from an oil-body-protein or an immunoglobulin, and the oil-body-protein can be an oleosin or a caleosin. The multimeric-fusion-protein can be a heteromultimeric-fusion-protein, wherein said first and second recombinant polypeptide form an enzymatically active heteromultimeric redox complex or an immunoglobulin. In a particular embodiment, the first recombinant polypeptide is a thioredoxin and the second recombinant polypeptide is a thioredoxin-reductase. The thioredoxin can be selected from the group consisting of SEQ ID NOs:38, 42, 46, 50 and SEQ ID NOs:52-194; and the thioredoxin-reductase can be selected from the group consisting of those set forth in SEQ ID NOs:8, 9, 10, 40, 44, 48, 50 and SEQ ID NOs:195-313. In another embodiment, the first recombinant polypeptide can be an immunoglobulin-polypeptide-chain. For example, the first recombinant polypeptide can be an immunoglobulin light chain, or an immunologically active portion thereof, and the second recombinant polypeptide can be an immunoglobulin heavy chain, or an immunologically active portion thereof. In this embodiment, the oil-body-targeting-protein can comprise protein A, protein L or protein G. In yet another embodiment, positioned between the nucleic acid sequence encoding an oil-body-targeting-protein and the nucleic acid sequence encoding a first recombinant polypeptide can be a linker nucleic acid sequence encoding [a] an oil-body-surface-avoiding linker amino acid sequence. The oil-



body-surface-avoiding linker amino acid sequence can be substantially negatively charged, or have a molecular weight of at least 35 kd. Optionally, the gene fusion further comprises a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by an enzyme or a chemical, wherein the linker sequence is positioned between the oil-body-surface-avoiding linker amino acid sequence and said sequence encoding the first recombinant polypeptide.

Also [provide] provided herein are isolated oil bodies comprising a multimeric-protein-complex comprising (i) an oil-body-targeting-protein and (ii) a first recombinant polypeptide, said oil bodies further comprising a second recombinant polypeptide, wherein said first and second recombinant polypeptide are capable of forming a multimeric-protein-complex. The oil-body-targeting-protein can be selected from an oil-body-protein or an immunoglobulin, and the oil-body-protein can be an oleosin or a caleosin. The multimeric-fusion-protein can be a heteromultimeric-fusion-protein, wherein said first and second recombinant polypeptide form an enzymatically active heteromultimeric redox complex or an immunoglobulin. In a particular embodiment, the first recombinant polypeptide is a thioredoxin and the second recombinant polypeptide is a thioredoxin-reductase. In another embodiment, the first recombinant polypeptide can be an immunoglobulin-polypeptide-chain. For example, the first recombinant polypeptide can be an immunoglobulin light chain, or an immunologically active portion thereof, and the second recombinant polypeptide can be an immunoglobulin heavy chain, or an immunologically active portion thereof. In this embodiment, the oil-body-targeting-protein can comprise protein A, protein L or protein G.

**Please amend the paragraph on page 10, line 25, to page 11, line 14, as follows;**

Also provided are cells and transgenic plants comprising oil bodies, multimeric-protein-complexes, and multimeric-fusion-proteins, set forth herein. In one embodiment, the first recombinant polypeptide can be an

immunoglobulin-polypeptide-chain. For example, the first recombinant polypeptide can be an immunoglobulin light chain, or an immunologically active portion thereof, and the second recombinant polypeptide can be an immunoglobulin heavy chain, or an immunologically active portion thereof. In this embodiment, the oil-body-targeting-protein can comprise protein A, protein L or protein G. In embodiments, wherein said first recombinant polypeptide is a thioredoxin and said second recombinant polypeptide is a thioredoxin-reductase, the methods described herein can be used to formulate the oil bodies for use in the preparation of a food product, personal care product or pharmaceutical composition. These formulations can further comprise the addition of NADP or NADPH. The food product can be a milk or wheat based food product. The personal care product can reduce the oxidative stress to the surface area of the human body or can be used to lighten the skin. The pharmaceutical composition can be used to treat chronic obstructive pulmonary disease (COPD), cataracts, diabetes, envenomation, bronchiopulmonary disease, malignancies, psoriasis, reperfusion injury, wound healing, sepsis, GI bleeding, intestinal bowel disease (IBD), ulcers, GERD (gastro esophageal reflux disease).

**Please amend the paragraphs on page 13, line 30, to page 14, line 20, as follows;**

c) isolating from said progeny cell said oil bodies comprising said redox fusion polypeptide or immunoglobulin. In certain embodiments, positioned between said nucleic acid sequence encoding a sufficient portion of an oil-body-protein and said nucleic acid sequence encoding a redox fusion polypeptide or immunoglobulin can be a linker nucleic acid sequence encoding [a] an oil-body-surface-avoiding linker amino acid sequence. The oil-body-surface-avoiding linker amino acid sequence can be substantially negatively charged or have a molecular weight of at least 35 kd. Optionally, the gene fusion further comprises a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by an enzyme or a chemical, wherein the linker sequence is positioned between the oil-body-surface-avoiding linker amino acid



sequence and said nucleic acid sequence encoding a redox fusion polypeptide. In this optional embodiment, also contemplated is the introduction of an enzyme or chemical that cleaves said redox fusion polypeptide from said oil body, thereby obtaining isolated redox fusion polypeptide. The first redox protein can be a thioredoxin and said second redox protein can be a thioredoxin-reductase. In one embodiment, the thioredoxin and thioredoxin-reductase can be obtained from *Arabidopsis*. In another embodiment, the first redox protein is at least 5 times more active when produced as a redox fusion polypeptide as compared to the production of the first redox protein without the second redox protein.

**Please amend the paragraphs on page 15, line 6, to page 16, line 5, as follows;**

3) a third nucleic acid sequence capable of terminating transcription in said cell. The oil-body-protein can be an oleosin or a caleosin, the first redox protein can be a thioredoxin and said second redox protein can be a thioredoxin-reductase. In certain embodiments, positioned between said nucleic acid sequence encoding a sufficient portion of an oil-body-protein and said nucleic acid sequence encoding a redox fusion polypeptide is a linker nucleic acid sequence encoding [a] an oil-body-surface-avoiding linker amino acid sequence. The oil-body-surface-avoiding linker amino acid sequence can be substantially negatively charged, or have a molecular weight of at least 35 kd. In one embodiment, the gene fusion optionally further comprises a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by an enzyme or a chemical, wherein the linker sequence is positioned between the oil-body-surface-avoiding linker amino acid sequence and said nucleic acid sequence encoding a redox fusion polypeptide.

Also provided herein are [transgenetic] transgenic plants, e.g., safflower plants, comprising any of the chimeric nucleic acid sequences and constructs described herein. The chimeric nucleic acids can be contained within a plastid. Accordingly, isolated plastids are provided having chimeric nucleic acids therein. Also provided are plant seeds comprising the chimeric nucleic acids provided herein.

Also provided are oil body preparations obtained using any of the methods provided herein, and food products, pharmaceutical compositions, and personal care products containing the oil body preparations. The products and/or compositions provided herein are capable of treating oxidative stress in a target, capable of chemically reducing a target. Also provided is a [detergent] detergent composition comprising an oil body preparation capable of chemically reducing a target, and related methods of cleansing an item, comprising administering such product to the item under conditions that promote cleansing. **Please amend the paragraphs on page 16, line 14, to page 18, line 4, as follows;**

In another embodiment, the at least one thioredoxin-related protein can be thioredoxin-reductase. The thioredoxin-reductase can be selected from the group consisting of those set forth in SEQ ID NOs:8, 9, 10, 40, 44, 48, 50 and SEQ ID NOs:195-313 and/or derived from *Arabidopsis* or wheat. The thioredoxin-reductase can be an NADPH-dependent thioredoxin-reductase. The second region can encode a thioredoxin and thioredoxin-reductase. In one embodiment, the thioredoxin and thioredoxin-reductase is obtained from *Mycobacterium leprae*. In another embodiment, the at least one thioredoxin-related protein can be an engineered fusion protein. The first region can precede, in a 5' to 3' direction, the second region. Alternatively, the first region follows, in a 5' to 3' direction, the second region. The gene fusion can optionally further comprise a third region encoding a second thioredoxin-related protein or an active fragment thereof, operably linked to the first region, or to the second region, or to both. A seed-specific promoter, such as a phaseolin promoter, can be operably linked to the gene fusion. In one embodiment, at least one thioredoxin-related protein is derived from a plant species selected from the group consisting of *Arabidopsis* and wheat. In another embodiment, at least one [thioreoxin-related] thioredoxin-related protein can be derived from *E. coli*.

In one embodiment, the gene fusion further comprises a nucleic acid

sequence encoding [a] an oil-body-surface-avoiding linker amino acid sequence, wherein the linker amino acid sequence is positioned between the first region and the second region. The oil-body-surface-avoiding linker amino acid sequence can be substantially negatively charged, or have a molecular weight of at least 35 kd. In addition, the gene fusion can further comprise a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by an enzyme or a chemical, wherein the linker sequence is positioned between the oil-body-surface-avoiding linker amino acid sequence and the second region.

Also provided herein are transgenic plants containing a nucleic acid construct comprising a gene fusion, wherein the gene fusion comprises a region encoding an oil-body-protein or an active fragment thereof, operably linked to a region encoding a first thioredoxin-related protein or an active fragment thereof. The thioredoxin-related protein can be thioredoxin. The nucleic acid construct can be contained within a plastid. In one embodiment, when the first thioredoxin-related protein is thioredoxin and the construct can further comprise a region encoding a thioredoxin-reductase. The gene fusion can optionally further comprise a third region encoding a second thioredoxin-related protein or an active fragment thereof, operably linked to the first region, or to the second region, or to both. The gene fusion can [further] optionally further comprise a nucleic acid sequence encoding [a] an oil-body-surface-avoiding linker amino acid sequence, wherein the nucleic acid encoding the linker amino acid sequence is positioned between the region encoding an oil-body-protein and the region encoding a first thioredoxin-related protein. The oil-body-surface-avoiding linker amino acid sequence can be substantially negatively charged, or have a molecular weight of at least 35 kd. The gene fusion can optionally further comprise a linker nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by an enzyme or a chemical, wherein the linker sequence is positioned between the oil-body-surface-avoiding linker amino acid sequence and the region encoding a first thioredoxin-related protein.

**Please amend the paragraph on page 24, lines 3-16, as follows;**

The recombinant polypeptide or multimeric-protein-complex is associated with an oil body. As used herein, the phrase "oil body" or "oil bodies" refers to any oil or fat storage organelle in any cell type. Accordingly, the oil bodies may be obtained from any cell comprising oil bodies, including plant cells (described in for example: Huang (1992) *Ann. Rev. Plant Mol. Biol.* 43: 177-200), animal cells (described in for example: Murphy (1990) *Prog Lipid Res* 29(4): 299-324), including adipocytes, hepatocytes, [steroigogenic] steroidogenic cells, mammary epithelial cells, macrophages, algae cells (described in for example: Rossler (1988) *J. Physiol. London*, 24: 394-400) fungal cells, including yeast cells (described in for example Leber et al. (1994) *Yeast* 10: 1421-1428) and bacterial cells (described in for example: Pieper-Furst et al. (1994) *J. Bacteriol.* 176: 4328-4337). Preferably the oil bodies used herein are oil bodies obtainable from plant cells and more preferably the oil bodies obtainable from plant seed cells.

**Please amend the paragraph on page 27, line 25, to page 28, line 7, as follows;**  
**[Multimeric-protein-complexes] Multimeric-protein-complexes**

In accordance with the methods and compositions provided herein, any two recombinant polypeptides capable of forming a multimeric-protein-complex may be used. The nucleic acid sequences encoding the two recombinant polypeptides may be obtained from any biological source or may be prepared synthetically. In general nucleic acid sequence encoding multimeric proteins are known to the art and readily available. Known nucleic acid sequences encoding multimeric-protein-complexes may be used to design and construct nucleic acid sequence based probes in order to uncover and identify previously undiscovered nucleic acid sequences encoding multimeric-protein-complexes, for example, by screening cDNA or genomic libraries or using 2- or multi-hybrid systems. Thus, additional nucleic acid sequences encoding multimeric-protein-complexes may be discovered and used as described herein.

**Please amend the paragraph on pag 34, lin 22-31, as follows;**

Oil-body-targeting-proteins may also be chemically modified. For

example, oleosins may be modified by changing chemical modification of the lysine residues using chemical agents such as [biotinyl-N-hydroxysuccinimide] biotinyl-N-hydroxysuccinimide ester resulting in a process referred to as biotinylation. Conveniently this is accomplished by *in vitro* biotinylation of the oil bodies. *In vivo* biotinylation may be accomplished using the biotinylation domain peptide from the biotin carboxy carrier protein of *E. coli* acetyl-CoA carboxylase (Smith et al. (1998) Nucl. Acids. Res. 26: 1414-1420). Avidin or streptavidin may subsequently be used to accomplish association of the redox protein with the oil body.

**Please amend the paragraph on page 36, line 3, to page 36, line 16, as follows;**

In a further embodiment both the first and second recombinant polypeptide are separately fused to an oil-body-protein. In this embodiment nucleic acid sequences encoding the first and second polypeptides may be prepared separately and introduced in separate cell lines or they may be introduced in the same cell lines. Where the nucleic acid sequences are introduced in the same cell line, these nucleic acid sequence may be prepared using two separate expression vectors, or they may be prepared using a single vector comprising nucleic acid sequences encoding both the first polypeptide fused to an oil body protein and the second polypeptide fused to an oil-body-protein. Where separate cell lines are used subsequent mating of the offspring (e.g., mating of plants) is used to prepare a generation of cells comprising oil bodies which comprise both the first and second recombinant polypeptide fused to an oil-body-protein.

**Please amend the paragraphs on page 37, line 26, to page 38, line 1, as follows;**

(a) producing in a cell comprising oil bodies, a first redox protein and a second redox protein wherein said first redox protein is capable of interacting with said second redox protein, preferably in the cell, to form said [heteromultimeric] heteromultimeric redox protein complex; and

(b) associating said heteromultimeric redox protein complex with an oil

body through an oil-body-targeting-protein capable of associating with said oil bodies and said heteromultimeric redox protein complex.

**Please amend the paragraph on page 38, line 14-27, as follows;**

As used herein the phrase "redox proteins" or grammatical variations thereof, refers to any protein or active protein fragment capable of participating in electron transport. For example, redox proteins are capable of catalyzing the transfer of an electron from an electron donor (also frequently referred to as the reducing agent) to an electron acceptor (also frequently referred to as the oxidizing agent). In the process of electron transfer, the reducing agent (electron donor) is oxidized and the oxidizing agent (electron acceptor) is reduced. Exemplary redox proteins for use herein include iron-sulfur proteins, cytochromes, redox active thiol proteins and redox-active flavoproteins. To carry out their function as conduits for electrons [donors], redox proteins, such as thioredoxin and thioredoxin-reductase for example, are known to function by interacting or associating with one another in multimeric-protein-complexes (e.g., heteromultimeric-protein-complexes).

**Please amend the paragraph on page 42, lines 8-20, as follows;**

First and second redox proteins that may be used herein include without limitation any first redox protein and second redox protein selected from the group of redox proteins consisting of cytochromes, such as cytochrome a, cytochrome b and cytochrome c; porphyrin containing proteins, for example [haemoglobin] hemoglobin; iron-sulfur proteins, such as ferredoxin; flavoproteins such as thioredoxin-reductase, NADH dehydrogenase, succinate dehydrogenase, dihydrolipoyl dehydrogenase, acyl-CoA dehydrogenase, D-amino acid oxidase, xanthine oxidase, orotate reductase and aldehyde oxidase; pyridine-linked dehydrogenases, for example, lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, and beta-hydroxy-butarate dehydrogenase; and redox active thiol containing proteins such as thioredoxin.

**Please amend the paragraph on pag 45, lin s 2-17, as follows;**

As used herein, the term "thioredoxin-reductase" refers to a protein that



complexes with a flavin, such as FAD. The flavin compound serves as an electron donor for the thioredoxin-reductase protein active site. Thioredoxin reductases have a redox active, disulfide bond site capable of reducing thioredoxin. The active site of thioredoxin-reductase contains 2 cysteines. The type of amino acids surrounding the 2 cysteine residues forming the active site can vary as hydrophobic, non-polar or polar. An exemplary thioredoxin-reductase is NADPH-thioredoxin-reductase [(TR)] (NTR), which is a cytosolic homodimeric enzyme comprising typically 300-500 amino acids. Crystal structures of both *E. coli* and plant thioredoxin-reductase have been obtained (Waksman et al. (1994) J. Mol. Biol. 236: 800-816; Dai et al. (1996) J. Mol. Biol. 264:1044-1057). NADPH-thioredoxin-reductases have been expressed in heterologous hosts, for example the *Arabidopsis* NADPH-thioredoxin-reductase has been expressed in *E. coli* (Jacquot et al. (1994) J. Mol. Biol. 235: 1357-1363) and wheat (PCT Patent Application 00/58453).

**Please amend the paragraph on page 46, lines 2-16, as follows;**

Also contemplated for use in the methods and compositions provided herein are nucleic acid and amino acid homologs that are "[substantially] substantially homologous" to the thioredoxin and thioredoxin-reductase nucleic and amino acids set forth herein, which includes thioredoxin and thioredoxin-reductase polypeptides encoded by a sequence of nucleotides that hybridizes under conditions of low, moderate or high stringency to the sequence of nucleotides encoding the thioredoxin and thioredoxin-reductase nucleic and amino acids set forth herein (e.g., in the Examples, Sequence Listing and/or Table 5). As used herein, a DNA or nucleic acid homolog refers to a nucleic acid that includes a preselected conserved nucleotide sequence, such as a sequence encoding a therapeutic polypeptide. By the term "substantially homologous" is meant having at least 80%, preferably at least 90%, most preferably at least 95% homology therewith or a less percentage of homology or identity and conserved biological activity or function.

**Please amend the paragraph on page 49, lines 6-16, as follows;**

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, *e.g.*, Sambrook, E.F. Fritsch, T. Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see, also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 [~~phophate~~ phosphate- buffered 0.18 NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by  $T_m$ , which is a function of the sodium ion concentration and temperature ( $T_m = 81.5^{\circ} \text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G} + \text{C}) - 600/l$ )), so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

**Please amend the paragraph on page 52, lines 7-31, as follows;**

In accordance with the present invention, the first and/or second recombinant polypeptides, multimeric-protein-complexes, heteromultimeric-protein-complexes, multimeric-fusion-proteins, heteromultimeric-fusion-proteins, immunoglobulins, immunoglobulin-polypeptide-chains, redox-fusion-polypeptides, or the first and/or second thioredoxin-related proteins; and the oil-body-targeting-protein are conveniently produced in a cell. In order to produce the recombinant polypeptides or multimeric-protein-complexes, a nucleic acid sequence encoding either [the] the first and/or second recombinant polypeptides, multimeric-protein-complexes, heteromultimeric-protein-complexes, multimeric-fusion-proteins, heteromultimeric-fusion-proteins, immunoglobulins, immunoglobulin-polypeptide-chains, redox-fusion-polypeptides, or the first and/or second thioredoxin-related proteins; and/or the oil-body-targeting-protein are incorporated in a recombinant expression vector. Accordingly, provided herein are recombinant expression vectors comprising the chimeric nucleic acids provided herein suitable for expression of the oil-body-targeting-protein and the first and/or second recombinant polypeptides, multimeric-protein-complexes, heteromultimeric-protein-complexes, multimeric-fusion-proteins, heteromultimeric-fusion-proteins, immunoglobulins,

immunoglobulin-polypeptide-chains, redox-fusion-polypeptides, or the first and/or second thioredoxin-related proteins, suitable for the selected cell. The term "suitable for expression in the selected cell" means that the recombinant expression vector contains all nucleic acid sequences required to ensure expression in the selected cell.

**Please amend the paragraph on page 56, line 20, to page 57, line 10, as follows;**

The recombinant expression vector further may contain a marker gene. Marker genes that may be used in accordance with the present invention include all genes that allow the distinction of transformed cells from non-transformed cells including all selectable and screenable marker genes. A marker may be a resistance marker such as an antibiotic resistance marker against for example kanamycin, ampicillin, G418, bleomycin hygromycin, chloramphenicol which allows selection of a trait by chemical means or a tolerance marker against for example a chemical agent such as the normally phytotoxic sugar mannose (Negrotto et al. (2000) Plant Cell Rep. 19: 798-803). In plant recombinant expression vectors herbicide resistance markers may conveniently be used for example as markers conferring resistance against glyphosate (US Patents 4,940,935 and 5,188,642) or phosphinothricin (White et al. (1990) Nucl. Acids Res. 18: 1062; Spencer et al. (1990) Theor. Appl. Genet. 79: 625-631). Resistance markers to a herbicide when linked in close proximity to the redox protein or oil-body-targeting-protein may be used to maintain selection pressure on a population of plant cells or plants for those plants that have not lost the protein of interest. Screenable markers that may be employed to identify transformants through visual observation include beta-glucuronidase (GUS) (see US Patents US5,268,463 and US5,599,670) and green fluorescent protein (GFP) (Niedz et al. (1995) Plant Cell Rep.: 14: 403).

**Please amend the paragraphs on page 61, lines 3-26, as follows;**

Plastid transformation is [decribed] described in US Patents 5,451,513; 5,545,817 and 5,545,818; and PCT Patent Applications 95/16783; 98/11235

and 00/39313) Basic chloroplast transformation involves the introduction of cloned plastid DNA flanking a selectable marker together with the nucleic acid sequence of interest into a suitable target tissue using for example biolistics or protoplast transformation. Selectable markers that may be used include for example the bacterial *aadA* gene (Svab et al. (1993) Proc. Natl. Acad. Sci. USA 90: 913-917). Plastid promoters that may be used include for example the tobacco clpP gene promoter (PCT Patent Application 97/06250).

In another embodiment, the invention chimeric nucleic acid [constructs] constructs provided herein are directly transformed into the plastid genome. Plastid transformation technology is described extensively in U.S. Patent Nos. 5,451,513, 5,545,817, 5,545,818 and 5,576,198; in PCT application nos. WO 95/16783 and WO 97/32977; and in McBride et. al., *Proc Natl Acad Sci USA* 91: 7301-7305 (1994), the entire disclosures of all of which are hereby incorporated by reference. In one embodiment, plastid transformation is achieved via biolistics, first carried out in the unicellular green alga *Chlamydomonas reinhardtii* (Boynton et al. (1988) *Science* 240:1534-1537)) and then extended to *Nicotiana tabacum* (Svab et al. (1990) *Proc Natl Acad Sci USA* 87:8526-8530), combined with selection for cis-acting antibiotic resistance loci (spectinomycin or streptomycin resistance) or complementation of non-photosynthetic mutant phenotypes.

**Please amend the paragraphs on page 67, lines 9-29, as follows;**

Following transformation the cells are grown, typically in a selective medium allowing the identification of transformants. Cells may be harvested in accordance with methodologies known to the art. In order to associate the oil bodies with the first and/or second recombinant polypeptides, multimeric-protein-complexes, heteromultimeric-protein-complexes, multimeric-fusion-proteins, heteromultimeric-fusion-proteins, immunoglobulins, immunoglobulin-polypeptide-chains, redox-fusion-polypeptides, and a first and/or second thioredoxin-related protein, the integrity of cells may be disrupted using any physical, chemical or biological methodology capable of disrupting the cells'

integrity. These methodologies are generally cell-type dependent and known to the skilled artisan. Where plants are employed they may be regenerated into mature plants using plant tissue culture techniques generally known to the skilled artisan. Seeds may be harvested from mature transformed plants and used to propagate the plant line. Plants may also be crossed and in this manner, contemplated herein is the [breedig] breeding of cells lines and transgenic plants that vary in genetic background. It is also possible to cross a plant line comprising the first recombinant polypeptide with a plant line comprising the second recombinant polypeptide. Accordingly, also provided herein are methods of producing in a plant a recombinant multimeric-protein-complex, said method comprising:

(a) preparing a first plant comprising cells, said cells comprising oil bodies and a first recombinant polypeptide, such as a redox protein (e.g., a thioredoxin-related protein, and the like) or an immunoglobulin-polypeptide-chain, wherein said first recombinant polypeptide is capable of associating with said oil bodies through an oil-body-targeting-protein;

**Please amend the paragraph on page 69, line 12, to page 70, line 3, as follows;**

In certain embodiments, the oil bodies are obtained from a plant cell such as for example a pollen cell; a fruit cell; a spore cell; a nut cell; mesocarp cell; for example the mesocarp cells obtainable from olive (*Olea europaea*) or avocado (*Persea americana*); or a seed cell. In particular embodiments the oil bodies are obtained from a plant seed cell. The seeds can be obtained from a transgenic plant according to the present invention. In particular embodiments, a seed of a transgenic plant according to the present invention contains the first and/or second recombinant polypeptides, multimeric-protein-complexes, heteromultimeric-protein-complexes, multimeric-fusion-proteins, heteromultimeric-fusion-proteins, immunoglobulins, immunoglobulin-polypeptide-chains, redox-fusion-polypeptides, or first and/or second thioredoxin-related proteins in a concentration of at least about 0.5% of total cellular seed protein. In further embodiments, a seed of a transgenic plant provided herein contains a



recombinant polypeptide or multimeric-protein-complex in a concentration of at least about 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0%, 2.25%, 2.5%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% or more, of total cellular seed protein. The upper limits of the recombinant polypeptide or multimeric-protein-complex concentration can be up to about 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%. Thus, the ranges at least about 0.5% up to about 15%; at least about 1.0% up to about 10%; and at least about 5% up to about 8% are [amoung] among the various ranges contemplated herein.

**Please amend the paragraph on page 70, line 26, to page 71, line 6, as follows;**

Seed grinding may be accomplished by any comminuting process resulting in a substantial disruption of the seed cell membrane and cell walls without compromising the structural integrity of the oil bodies present in the seed cell. Suitable grinding processes in this regard include mechanical pressing and milling of the seed. Wet milling processes such as [decribed] described for cotton (Lawhon et al. (1977) J. Am. Oil Chem. Soc. 63: 533-534) and soybean (US Patent 3,971,856; Carter et al. (1974) J. Am. Oil Chem. Soc. 51: 137-141) are particularly useful in this regard. Suitable milling equipment capable of industrial scale seed milling include colloid mills, disc mills, pin mills, orbital mills, IKA mills and industrial scale homogenizers. The selection of the milling equipment will depend on the seed, which is selected, as well as the throughput requirement.

**Please amend the paragraph on page 75, lines 11-27, as follows;**

The first recombinant polypeptide, second recombinant polypeptide and oil-body-targeting-protein may also be prepared in separate cellular compartments. Association of the first polypeptide, second polypeptide, and oil body then may occur upon disruption of the cell's integrity. For [exmples] example, various mechanisms for targeting gene products are known to exist in plants, and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a transit sequence found at the amino terminal



end of various proteins which is cleaved during chloroplast import to yield the mature protein (Comai *et al.* (1988) *J Biol Chem* 263: 15104-15109). Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (Unger *et al.* (1989) *Plant Mol Biol* 13:411-418). The cDNAs encoding these products can be manipulated to target heterologous gene products to these organelles. In addition, sequences have been characterized which cause the targeting of gene products to other cell compartments.

**Please amend the paragraph on page 84, line 31, to page 85, line 22, as follows;**

The composition can contain along with the active ingredient: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acaciagelatin, glucose, molasses, [polvinylpyrrolidone] polivinylypyrrolidone, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents. Methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art (see, *e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975). The composition or formulation to be administered will contain a quantity of the active compound in an amount sufficient to alleviate the symptoms of the treated subject.

Please amend the paragraphs on page 91, line 14, to page 95, line 27, as follows;

***Construction of plant transformation vector pSBS2520.***

The *Arabidopsis* thioredoxin h gene as described in example 1 was placed under the regulatory control of the phaseolin promoter and the phaseolin terminator derived from the common bean *Phaseolus vulgaris* (Slightom et al (1983) Proc. Natl Acad Sci USA 80: 1897-1901; Sengupta-Gopalan *et al.*, (1985) PNAS USA 82: 3320-3324)). A gene splicing by overlap extension technique (Horton et al (1989) 15: 61-68) was used to fuse the phaseolin promoter to the Trxh gene. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were used to furnish the phaseolin promoter and terminator with Pst I and HindIII/KpnI sites respectively (see SEQ ID NO:14). Standard molecular biology laboratory techniques were also used to place the phaseolin terminator [downstream] downstream from the Trxh gene. The PstI-phaseolin promoter- Trxh-phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000 (pSBS3000 is a derivative from the *Agrobacterium* binary plasmid pPZP221 (Hajdukiewicz et al., 1994, Plant Molec. Biol. 25: 989-994). In pSBS3000, the CaMV35S promoter-gentamycin resistance gene-CAMV 35S terminator of pPZP221 was replaced with parsley ubiquitin promoter-phosphinothricin acetyl transferase gene-parsley ubiquitin termination sequence to confer resistance to the herbicide glufosinate ammonium.) The resulting plasmid is called pSBS2520. The sequence of the phaseolin promoter-*Arabidopsis* Trxh-phaseolin terminator sequence is shown in SEQ ID NO:14.

***Construction of plant transformation vector pSBS2510.***

The 3' coding sequence of an *Arabidopsis* oleosin gene (van Rooijen et al (1992) Plant Mol. Biol. 18: 1177-1179) was altered to contain an NcoI site. The NcoI-HindIII fragment from vector pSBS2500 (Example 1) containing the Trxh was ligated to the coding sequence of this *Arabidopsis* oleosin utilizing this NcoI restriction site. A gene splicing by overlap extension technique (Horton et al

(1989) 15: 61-68) was used to fuse the phaseolin promoter (Slightom et al (1983) Proc. Natl Acad Sc USA 80: 1897-1901; Sengupta-Gopalan *et al.*, (1985) PNAS USA 82: 3320-3324) containing a synthetic PstI site (see construction of pSBS2520) to the coding sequence of the *Arabidopsis* oleosin. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were again used to clone the HindIII KpnI fragment containing the phaseolin terminator (see construction of pSBS2520) [downstream] downstream of the Trxh gene. The PstI-phaseolin promoter- oleosin- Trxh-phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000. The resulting plasmid is called pSBS2510. The sequence of the phaseolin promoter-oleosin Trxh-phaseolin terminator sequence is shown in SEQ ID NO:16.

***Construction of plant transformation vector pSBS2521.***

This vector contains the same genetic elements as the insert of pSBS2510 except the Trxh gene is fused to the 5' end of the oleosin gene. The 3' oleosin coding sequence including its native stopcodon (van Rooijen et al (1992) Plant Mol. Biol. 18: 1177-1179) was furnished with a HindIII cloning site. Again a gene splicing by overlap extension technique (Horton et al (1989) 15: 61-68) was used to fuse the phaseolin promoter to the Trxh gene and to fuse the Trxh gene to the oleosin sequence. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were again used to clone the HindIII KpnI fragment containing the phaseolin terminator (see construction of pSBS2520) [downstream] downstream of the oleosin gene. The PstI-phaseolin promoter- Trxh oleosin- phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000. The resulting plasmid is called pSBS2521. The sequence of the phaseolin promoter- Trxh oleosin -phaseolin terminator sequence is shown in SEQ ID NO:19.

***Construction of plant transformation vector pSBS2527.***

The *Arabidopsis* NADPH thioredoxin-reductase gene as described in example 1

was placed under the regulatory control of the phaseolin promoter and the phaseolin terminator derived from the common bean *Phaseolus vulgaris* (Slightom et al (1983) Proc. Natl Acad Sc USA 80: 1897-1901; Sengupta-Gopalan *et al.*, (1985) PNAS USA 82: 3320-3324). A gene splicing by overlap extension technique (Horton et al (1989) 15: 61-68) was used to fuse the phaseolin promoter to the thioredoxin-reductase gene. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were used to furnish the phaseolin promoter and terminator with PstI and HindIII/KpnI sites respectively (see SEQ ID NO:14). Standard molecular biology laboratory techniques were also used to place the phaseolin terminator [downstream] downstream from the thioredoxin-reductase gene. The PstI-phaseolin promoter-thioredoxin-reductase-phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000. The resulting plasmid is called pSBS2527. The sequence of the phaseolin promoter-*Arabidopsis* thioredoxin-reductase-phaseolin terminator sequence is shown in SEQ ID NO:22.

***Construction of plant transformation vector pSBS2531.***

A gene splicing by overlap extension technique (Horton et al (1989) 15: 61-68) was used to fuse the phaseolin promoter (Slightom et al (1983) Proc. Natl Acad Sc USA 80: 1897-1901; Sengupta-Gopalan *et al.*, (1985) PNAS USA 82: 3320-3324) to the coding sequence of the *Arabidopsis* oleosin. The same gene splicing technique was used to fuse the oleosin gene to the thioredoxin-reductase coding sequence. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were again used to clone the HindIII KpnI fragment containing the phaseolin [downstream] downstream of the thioredoxin-reductase gene. The PstI-phaseolin promoter- oleosin- thioredoxin-reductase -phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000. The resulting plasmid is called pSBS2531. The sequence of the phaseolin promoter-oleosin thioredoxin-reductase -phaseolin terminator sequence is shown in SEQ

ID NO:24.

***Construction of plant transformation vector pSBS2529***

This vector contains the same genetic elements as the insert of pSBS2531 except the thioredoxin-reductase gene is fused to the 5' end of the oleosin gene. The 3' oleosin coding sequence including its native stopcodon (van Rooijen et al. (1992) Plant Mol. Biol.18: 1177-1179) was furnished with a HindIII cloning site. Again a gene splicing by overlap extension technique (Horton et al (1989) 15: 61-68) was used to fuse the phaseolin promoter to the thioredoxin-reductase gene and to fuse the thioredoxin-reductase gene to the oleosin sequence. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were again used to clone the HindIII KpnI fragment containing the phaseolin terminator (see construction of pSBS2520) [downstream] downstream of the oleosin gene. The PstI-phaseolin promoter- thioredoxin-reductase oleosin-phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000. The resulting plasmid is called pSBS2529. The sequence of the phaseolin promoter- thioredoxin-reductase oleosin -phaseolin terminator sequence is shown in SEQ ID NO:27.

***Construction of plant transformation vector pSBS2530.***

A plant transformation was constructed containing the *Mycobacterium Leprae* thioredoxin-reductase /thioredoxin gene (*Mlep* TR/Trxh). A construct called pHIS/TR/Trxh (Wieles et al (1995) J Biol Chem 270:25604-25606) was obtained from the department of Immunohematology and Blood bank, Leiden University, The Netherlands and use as a template for PCR to generate pSBS2530. The construction of pSBS2530 was identical to the construction of pSBS2531 except that the *Mlep* TR/Trxh gene was used instead of the *Arabidopsis* thioredoxin-reductase gene. A gene splicing by overlap extension technique (Horton et al (1989) 15: 61-68) was used to fuse the phaseolin promoter (Slightom et al (1983) Proc. Natl Acad Sc USA 80: 1897-1901; Sengupta-Gopalan *et al.*, (1985) PNAS USA 82: 3320-3324) to the coding



sequence of the *Arabidopsis* oleosin. The same gene splicing technique was used to fuse the oleosin gene to the *Mlep* TR/Trxh coding sequence. Standard molecular biology laboratory techniques (see eg: Sambrook *et al.* (1990) Molecular Cloning, 2<sup>nd</sup> ed. Cold Spring Harbor Press) were again used to clone the HindIII-KpnI fragment containing the phaseolin [dowstream] downstream of the *Mlep* TR/Trxh gene. The PstI-phaseolin promoter- oleosin- *Mlep* TR/Trxh - phaseolin terminator-KpnI insert sequence was cloned into the PstI-KpnI sites of pSBS3000. The resulting plasmid is called pSBS2530. The sequence of the phaseolin promoter-oleosin *Mlep* TR/Trxh -phaseolin terminator sequence is shown in SEQ ID NO:30.

**Please amend the paragraph on page 97, lines 10-22, as follows;**

The *Arabidopsis* thioredoxin and thioredoxin-reductase genes were cloned in frame in bacterial expression vector pRSETB ([Invitrogen] Invitrogen) to allow for the overexpression of *Arabidopsis* thioredoxin and thioredoxin-reductase proteins. These proteins were purified using standard protocols (see eg Invitrogen protocol) and used to raise antibodies in rabbits using standard biochemical techniques (See eg Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989). The *Arabidopsis* oleosin gene genes was cloned in frame in bacterial expression vector pRSETB (Invitrogen) to allow for the overexpression *Arabidopsis* oleosin protein. This protein was purified using standard protocols (see eg Invitrogen protocol) and used to prepare mouse monoclonal antibodies using standard biochemical techniques (See eg Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989).

**Please amend the paragraphs on page 98, line 27, to page 100, line 13, as follows;**

Total seed and [oilbody] oil body protein extracts from plants transformed with pSBS25121 were loaded onto polyacrylamide gels and either stained with Coomassie brilliant blue or electroblotted onto PVDF membranes. The membranes were challenged with [with] a polyclonal antibody raised against *Arabidopsis* thioredoxin, or a monoclonal antibody raised against the *Arabidopsis*



18.5 kDa oleosin and [and] visualized using alkaline phosphatase. Expression of the thioredoxin-oleosin results in an additional band of 31.2 kDa. The results indicate that the thioredoxin antibodies are immunologically reactive with a band of the right predicted molecular weight (31.2 kDa), and the oleosin antibodies are also immunologically reactive with a band of the right predicted molecular weight for the fusion protein (31.2 kDa) in addition to a band corresponding to the native *Arabidopsis* oleosin (18.5 kDa). This indicates that thioredoxin-oleosin is expressed in *Arabidopsis* seeds and is correctly targeted to [oilbodies] oil bodies.

***Analysis of seed extracts from plants transformed with pSBS2520*** Total seed extracts from plants transformed with pSBS2520 were loaded onto polyacrylamide gels and either stained with Coomassie brilliant blue or electroblotted onto PVDF membranes. The membranes were challenged with [with] a polyclonal antibody raised against *Arabidopsis* thioredoxin and visualized using alkaline phosphatase. The results indicated that the thioredoxin antibodies are immunologically reactive with a band of approximately the right predicted molecular weight (12 kDa). Untransformed seeds do not show a detectable thioredoxin band.

***Analysis of seed and oil body extracts from plants transformed with pSBS2529***

Total seed and oilbody protein extracts from plants transformed with pSBS2529 were loaded onto polyacrylamide gels and electroblotted onto PVDF membranes. The membranes were challenged with [with] a polyclonal antibody raised against *Arabidopsis* thioredoxin-reductase, or a monoclonal antibody raised against the *Arabidopsis* 18.5 kDa oleosin and [and] visualized using alkaline phosphatase. Expression of the thioredoxin-reductase-oleosin results in an additional band of 53.8 kDa. The results indicate that the thioredoxin-reductase antibodies are immunologically reactive with a band of the right predicted molecular weight for the fusion protein (53.8 kDa), the oleosin antibodies are also immunologically reactive with a band of the right predicted molecular weight (53.8 kDa) in

addition to a band corresponding to the native *Arabidopsis* oleosin (18.5 kDa). This indicates that thioredoxin-reductase-oleosin is expressed in *Arabidopsis* seeds.

***Analysis of seed extracts from plants transformed with pSBS2527***

Total seed extracts from plants transformed with pSBS2527 were loaded onto polyacrylamide gels and electroblotted onto PVDF membranes. The membranes were challenged with [with] a polyclonal antibody raised against *Arabidopsis* thioredoxin-reductase and visualized using alkaline phosphatase. The thioredoxin-reductase antibodies are immunologically reactive with a band of approximately the right predicted molecular weight for the (35.3 kDa). Untransformed seeds do not show a detectable thioredoxin band.

**Please amend the paragraph on page 101, lines 23-30, as follows;**

The activity of the thioredoxin and [thioredoxin] thioredoxin reductase was determined using a colorimetric DTNB [5,5'-dithiolbis (2-nitrobenzoic acid)] assay. The assay was performed in a 700  $\mu$ L reaction volume containing 100mM Tris-Cl pH 8.0, 5 mM EDTA, 200 $\mu$ M DTNB [5,5'-dithiolbis (2-nitrobenzoic acid)] and 200 $\mu$ M NADPH. If thioredoxin-reductase and thioredoxin are added, NADPH will reduce the thioredoxin-reductase, which will then reduce thioredoxin, which will, in turn, reduce DTNB (see equations below).

**Please amend the paragraph on page 113, lines 10-12, as follows;**

**Example 6**

**Production of assembled multimeric-immunoglobulin-[complexess]complexes as fusions with oil body targeting domains.**

**Please amend the paragraph on page 115, lines 5-8, as follows;**

SEQ ID NOs:5-7 set forth primers which were designed to code for a specific linker peptide between thioredoxin reductase and thioredoxin proteins, as described in [Exmaple] Example 2.

**Please amend the paragraph on page 115, lines 11-13, as follows;**

SEQ ID NOs:9 and 11, [respectivley] respectively, set forth the nucleotide sequence of the published NADPH thioredoxin reductase sequence (ATTHIREDB)

and the deduced amino acid sequence.

**Please amend the paragraph on page 117, lines 4-13, as follows;**

SEQ ID NOs:27, 28 and 29 show the nucleotide sequence of the phaseolin promoter - thioredoxin-reductase oleosin - phaseolin terminator sequence as described in Example 2, and the deduced amino [acid] acid sequences. The thioredoxin-reductase coding sequence and its deduced amino acid sequence is indicated. The phaseolin promoter corresponds to nucleotide 6-1554. The thioredoxin-reductase coding sequence corresponds to nt 1555-2553. The sequence encoding oleosin corresponds to nt 2554-3315, the intron in this sequence (nt 2751-3146) is indicated in italics. The phaseolin terminator corresponds to nucleotide sequence 3321-4540.

**Please amend the paragraph on page 118, lines 15-19, as follows;**

SEQ ID NOs:48 and 49, set forth the nucleotide sequence of Human Thioredoxin [Reductase] Reductase and the encoded protein, respectively.

\* SEQ ID NOs:50 and 51, [respectively] respectively, set forth the nucleotide sequence of Mycobacterium leprae Thioredoxin-Thioredoxin [Reductase] Reductase and the encoded protein, respectively.

**IN THE CLAIMS:**

Please amend the claims as follows (deletions are [bracketed], and additions are underlined):

**Please amend claims 3 and 17 as follows:**

3. (Amended) A method of producing in a plant a recombinant multimeric-protein-complex, said method comprising:

(a) preparing a first plant comprising cells, said cells comprising oil bodies and a first recombinant polypeptide wherein said first recombinant polypeptide is capable of associating with said oil bodies through an oil-body-targeting-protein;

(b) preparing a second plant comprising cells, said cells comprising oil bodies and a second recombinant polypeptide; and

(c) sexually crossing said first plant with said second plant to produce a progeny plant comprising cells, said cells comprising oil bodies, wherein said oil

bodies are capable of associating with said first recombinant polypeptide, and said first recombinant [recombinant] polypeptide is capable of associating with said second recombinant polypeptide to form said recombinant multimeric-protein-complex,


wherein said first recombinant polypeptide is a thioredoxin and said second recombinant polypeptide is a thioredoxin-reductase.

17. (Amended) A [transgenenic] transgenic plant comprising the chimeric nucleic acid sequence of claim 16.

\* \* \*

Respectfully submitted,  
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